NIH Public Access
Author Manuscript
Toxicol Appl Pharmacol. Author manuscript; available in PMC 2009 April 15.

Published in final edited form as:
Toxicol Appl Pharmacol. 2008 April 15; 228(2): 135–143.

Involvement of Mitogen-activated Protein Kinases and NFκB in LPS-induced CD40 Expression on Human Monocytic Cells

Weidong Wu[a,b*], **Neil E. Alexis**[a,b], **Xian Chen**[d], **Philip A. Bromberg**[a,c], and **David B. Peden**[a,b,c]

a*Center for Environmental Medicine, Asthma, and Lung Biology, University of North Carolina, Chapel Hill, North Carolina 27599*

b*Department of Pediatrics, University of North Carolina, Chapel Hill, North Carolina 27599*

c*Medicine, University of North Carolina, Chapel Hill, North Carolina 27599*

d*Biochemistry and Biophysics and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599*

Abstract

CD40 is a costimulatory molecule linking innate and adaptive immune responses to bacterial stimuli, as well as a critical regulator of functions of other costimulatory molecules. The mechanisms regulating lipopolysaccharide (LPS)-induced CD40 expression have not been adequately characterized in human monocytic cells. In this study we used a human monocytic cell line, THP-1, to investigate the possible mechanisms of CD40 expression following LPS exposure. Exposure to LPS resulted in a dose- and time-dependent increase in CD40 expression. Further studies using immunoblotting and pharmacological inhibitors revealed that mitogen-activated protein kinases (MAPKs) and NFκB were activated by LPS exposure and involved in LPS-induced CD40 expression. Activation of MAPKs was not responsible for LPS-induced NFκB activation. TLR4 was expressed on THP-1 cells and pretreatment of cells with a Toll-like receptor 4 (TLR4) neutralizing antibody (HTA125) significantly blunted LPS-induced MAPK and NFκB activation and ensuing CD40 expression. Additional studies with murine macrophages expressing wild type and mutated TLR4 showed that TLR4 was implicated in LPS-induced ERK and NFκB activation, and CD40 expression. Moreover, blockage of MAPK and NFκB activation inhibited LPS-induced TLR4 expression. In summary, LPS-induced CD40 expression in monocytic cells involves MAPKs and NFκB.

Keywords

Lipopolysaccharide; CD40; Toll-like receptor; Mitogen-activated protein kinase

*Address correspondence to: Weidong Wu, Center for Environmental Medicine, Asthma and Lung Biology, University of North Carolina at Chapel Hill, North Carolina 27599, Tel. 919 843-2714; Fax. 919 966-9863; E-mail: Weidong_Wu@med.unc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Abbreviations: LPS, lipopolysaccharide; ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; MAPKs, mitogen-activated protein kinases; JNK, c-Jun NH2-terminal kinase; NFκB, nuclear factor κB; TLR, toll-like receptor; DC, dendritic cell; MFI, mean fluorescence intensity; STAT, signal transducers and activators of transcription

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Introduction

CD40 is a 45–50 kDa cell surface receptor of 277 amino acids, which is a member of the tumor necrosis factor (TNF) receptor superfamily. It was first identified and functionally characterized on B lymphocytes. Recent studies have revealed that CD40 is also expressed on other cell types, including monocytes, macrophages, fibroblasts, dendritic cells, endothelial cells, carcinoma cells, and epithelial cells (Banchereau *et al.*, 1994; van Kooten and Banchereau, 1997). CD40 ligand (CD40L) is expressed mainly and transiently on activated CD4+ T cells. Upon CD40-CD40L contact, upregulation of various cell surface molecules, such as class II major histocompatibility complex (MHC), CD80, and CD86, as well as the production of numerous cytokines and chemokines, such as interleukin-1 (IL-1), IL-6, IL-10, TNFα, and macrophage inflammatory protein1 (MIP-1) and cytotoxic radicals ensue (Schonbeck and Libby, 2001). The interaction between CD40 and CD40L promotes B-cell growth, differentiation, and immunoglobulin class switching, and facilitates maturation of dendritic cells and antigen-presentation (Banchereau *et al.*, 1994; Van Kooten and Banchereau, 1996; van Kooten and Banchereau, 2000). CD40 expression on non-hematopoietic cells might be relevant to inflammatory responses. In addition, both CD40 and CD40L have been detected in a natural soluble form, and could act at distinct sites (Hock *et al.*, 2006). CD40 has been implicated in many human diseases, particularly inflammatory autoimmune diseases (Benveniste *et al.*, 2004 ; Park *et al.*, 2007).

The CD40 gene is mapped to human chromosome 20q11–q13 and is expressed as a single 1.5-kb mRNA species. Cytokines and environmental stimuli have been shown to induce CD40 expression in a variety of cell types (Becker and Soukup, 2003; He *et al.*, 2007; Lee *et al.*, 2007; Miyazawa *et al.*, 2007). Distinct transcription factors including signal transducers and activators of transcription 1 (STAT1), Sp1, and nuclear factor κB (NFκB) and *cis*-elements such as Ets and Gas elements have been reported to participate in the transcriptional regulation of CD40 expression in murine macrophages and microglia (Nguyen and Benveniste, 2000; Tone *et al.*, 2002; Qin *et al.*, 2005; Qin *et al.*, 2006). Human and murine CD40 shares 62% amino acid identity. LPS-induced expression of CD40 and other co-stimulatory molecules has been detected on human monocyte-derived dendritic cells (Ardeshna *et al.*, 2000; Nakahara *et al.*, 2004). Although the downstream signal transduction and function of CD40 has been extensively studied in a variety of cell types, the upstream events that regulate CD40 gene expression have been less examined in human monocytic cells. In this study, a human monocytic cell line, THP-1, was used to examine the regulation of CD40 expression on LPS-treated cells. Involvement of MAPKs and NFκB signaling pathways were examined in LPS-induced CD40 expression. In addition, a TLR4 neutralizing antibody and TLR4-deficient mouse macrophage cell lines were also employed to investigate the role TLR4 played in LPS-induced CD40 expression. It was found that LPS-induced CD40 expression was regulated by TLR4-mediated activation of MAPKs and NFκB. Moreover, activated MAPKs and NFκB could further mediate TLR4 expression induced by LPS.

Methods

Materials and Reagents

LPS (*Escherichia coli* 0127:B8) was purchased from Sigma-Aldrich Co. (St. Louis, MO). SDS-PAGE supplies such as molecular mass standards and buffers were from Bio-Rad (Richmond, CA). The mouse antibodies CD40-Phycoerythrin (PE) and IgG1-PE were obtained from Beckman Coulter-Immunotech (Marseille, France). Rat anti-mouse CD40, mouse anti-human Toll-like receptor 4 (TLR4) antibodies and IgG2a were purchased from eBiosience (San Diego, CA). Phospho-specific and pan antibodies against extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), p38, and IκBα antibodies were obtained from Cell Signaling Technology (Beverly, MA).β-actin antibody was purchased from USBiological (Swampscott,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

MA). NFκB p65 and p52 supershift antibodies, TLR4 neutralizing antibody (HTA125), horseradish peroxidase (HRP)-conjugated goat anti-rabbit or goat anti-mouse IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The ERK kinase inhibitor U0126, the p38 kinase inhibitor SB203580, the JNK inhibitor SP600125, the proteasome inhibitor MG132, and the NFκB activation inhibitor Bay 11–7082 were purchased from EMD Biosciences (San Diego, CA).

Cell Culture

The human monocytic leukemia cell line THP-1 was purchased from ATCC (Rockville, USA). THP-1 cells were cultured in RPMI 1640 medium (Invitrogen, Grand Island, NY) containing 10% fetal bovine serum and 100 μg/ml penicillin/streptomycin at 37 °C in 5% CO_2. Cells were incubated in ultra low attachment plates (Corning Inc., Corning, NY) for LPS stimulation studies.

The murine macrophage cell lines HeNC2 expressing wild type TLR4, and the GG2EE expressing mutated TLR4 (LPS-hyporesponsive) were kindly provided by Dr. Steven B. Mizel (Wake Forest University, NC) (Mizel *et al.*, 2003). Cells were incubated in RPMI 1640 medium containing 10% fetal bovine serum and 100 μg/ml penicillin/streptomycin at 37 °C in 5% CO_2.

Immunoblotting

Cells with or without pretreatment of pharmacological inhibitors or neutralizing antibody were treated with LPS, washed twice with cold phosphate-buffered saline (PBS), and then lysed in RIPA buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitors: 20 μg/ml leupeptin, 20 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 200 μM sodium orthovanadate, and 20 mM sodium fluoride). Supernatants of cell lysates were subjected to SDS-PAGE, as described before (Wu *et al.*, 2005). Proteins were transferred onto nitrocellulose membrane. Membrane was blocked with 5% nonfat milk, washed briefly, incubated with primary antibody at 4°C overnight, followed by incubating with corresponding HRP-conjugated secondary antibody for 1 h at room temperature. Immunoblot images were detected using chemiluminescence reagents and the Gene Gynome Imaging System (Syngene, Frederick, MD).

Flow Cytometry

Cells with or without pretreatment of pharmacological inhibitors were stimulated with LPS for 24 h. Flow cytometry was performed with a FACSORT (Becton Dickinson, Miami, FL) by using an Argon-ion laser (wavelength 488nm) (Alexis *et al.*, 2005). The FACSORT was calibrated with Calibrate beads before each use, and 10,000 events were counted for all sample runs. PE-conjugated non-specific antibodies of the same isotype as the receptor antibodies were used as controls to establish background fluorescence and non-specific antibody binding. The (arithmetic) mean fluorescence intensity (MFI) of the cells stained with control antibody was subtracted from the MFI of cells stained with receptor antibodies to provide a measure of receptor-specific MFI. Relative cell size and density/granularity were quantified by analyzing light-scatter properties using Cell Quest software (Becton Dickinson), namely forward scatter for cell size and side scatter for density/granularity, and recording the mean fluorescence intensities for each.

Extraction of Nuclear Proteins and Electrophoretic Mobility Shift Assay (EMSA)

THP-1 cells with or without pretreatment of pharmacological inhibitors were stimulated with 1000 ng/ml LPS for 1 h. 100 ng/ml TNFα was used as a positive control. Cell pellets were washed twice with cold-PBS. Nuclear proteins were extracted with a Nuclear/Cytosol

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Fractionation kit (Biovision Inc., Mountain View, CA). EMSA assay was conducted using an EMSA Gel-Shift kit (Panomics Inc., Fremont, CA) according to the manufacture's instruction. Briefly, nuclear proteins were incubated with 2 μl of 5×binding buffer, 1 μl of poly d(I-C) (1 μg/ml), 1 μl of cold unlabeled or biotin-labeled NFκB probe (10 ng/ml), and 5 μl of distilled water at 18°C for 30 min. For gel supershift assay, 2 μl of concentrated p65 or p50 antibody was added into the reaction mixture, respectively, and incubated at room temperature for 10 min. After mixed with 1 μl of loading dye, the samples were subjected to electrophoresis in 0.5×TBE buffer. The protein-oligos complexes were transferred onto nylon membrane. The images were developed using the detection buffer and substrates provided by the manufacture, and detected using chemiluminescence reagents and the Gene Gynome Imaging System as described previously. The sequence of oligonucleotide probe for NFκB binding site is as follows: 5'-AGTTGAGGGGACTTTCCCAGGC-3'.

Statistics

Differences in CD40 expression were evaluated using nonparametric paired *t* tests with the overall α level set at 0.05. Data were presented as means ± SEMs unless otherwise noted.

Results

LPS Exposure Induced CD40 Expression on THP-1 Cells

In this study, we examined CD40 expression on human THP-1 cells exposed to LPS. Exposure of THP-1 cells to 1000ng/ml LPS for 24 h did not result in significant alteration in cell viability, as assessed by assay of lactate dehydrogenase (LDH) activity (Data not shown). As shown in Figure 1A, LPS exposure (10–1000 ng/ml) induced a dose-dependent increase in CD40 expression at 24 h. At 1000 ng/ml, LPS stimulated CD40 expression in a time-dependent fashion (Figure 1B). In summary, LPS challenge elevated CD40 expression on the surface of THP-1 cells.

LPS Induced Phosphorylation of MAPKs in THP-1 Cells

To explore the mechanisms underlying LPS-induced CD40 expression, the involvement of mitogen-activated protein kinases (MAPKs) including ERK, JNK, and p38 kinase were investigated in this study. Activation of MAPKs occurs through phosphorylation of specific residues of these kinases. To determine the activation of MAPKs in THP-1 cells, phosphorylation of MAPKs was measured using phospho-specific antibodies. THP-1 cells were treated with 1000 ng/ml LPS for 0, 30, 60, 120, and 240 min. Cell lysates were subjected to immunoblotting. As shown in Figure 2, LPS induced a time-dependent phosphorylation of ERK, JNK, and p38 kinase detectable as early as 30 min. LPS-induced JNK phosphorylation decreased at 4 h exposure of LPS. In summary, LPS exposure activated all three MAPKs in THP-1 cells.

Involvement of MAPKs in LPS-induced CD40 Expression

MAPKs play important roles in regulating gene expression and immunological responses (Su and Karin, 1996). Involvement of MAPKs in downstream signals of CD40 activation has been reported (van Kooten and Banchereau, 2000). In this study, we investigated whether activation of MAPKs participated in regulation of LPS-induced CD40 expression. Thus, selective kinase inhibitors including U0126, SP600125, and SB203580, which specifically inhibited ERK kinase, JNK, and p38 kinase activities, respectively, were employed to pretreat THP-1 cells prior to LPS stimulation (1000 ng/ml) for 24 h. In comparison to vehicle treatment (DMSO), all three MAPK inhibitors blunted LPS-induced CD40 expression (Figure 3). Among them, U0126 appeared to have a strongest inhibitory effect. In a whole, these data demonstrate that ERK, JNK, and p38 kinase are involved in LPS-induced CD40 expression.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Activation of NFκB Was Required for LPS-induced CD40 Expression

NFκB transcription factors are present in the cytoplasm in an inactive state, complexed with inhibitory IκB proteins, which cover the nuclear localization structures of NFκB. The function of NFκB is primarily regulated by IκB family members. Phosphorylation of cytoplasmic IκBα at two conserved NH2-terminal serine residues (Ser32 and Ser36) and its subsequent proteosomal degradation by the 26S proteasome allows NFκB nuclear translocation and activation of NFκB-dependent transcriptional activity (Wang *et al.*, 2002). To examine whether NFκB was activated in THP-1 cells exposed to LPS, phosphorylation of IκBα and IκBα degradation were determined. THP-1 cells were pretreated with the proteasome inhibitor MG-132, which prevents IκBα degradation by the 26S proteasome and, thereby, permits the accumulation of phosphorylated IκBα in stimulated cells (Wu *et al.*, 2002). Pretreated THP-1 cells were stimulated with 1000 ng/ml LPS. Cytoplasmic protein fractions were separated by SDS-PAGE and immunoblotted with a phospho-specific antibody against the Ser32-phosphorylated form of IκBα. As shown in Figure 4A, LPS stimulation increased IκBα phosphorylation. Consistently, IκBα degradation was observed within the same exposure periods (Figure 4B). Therefore, these data suggested that LPS exposure induced NFκB activation in THP-1 cells.

To further demonstrate whether NFκB activation was involved in LPS-induced CD40 expression on THP-1 cells, a potent NFκB activation inhibitor, Bay 11–7082, was employed to pretreat cells prior to LPS treatment for 24 h as described previously (Shanmugam *et al.*, 2003). The cell viability following Bay 11–7082 treatment was determined by trypan blue dye exclusion assay, which showed that more than 90% of THP-1 cells were viable. As shown in Figure 4C, Bay 11–7082 significantly suppressed LPS-induced CD40 expression in a dose-dependent fashion on THP-1 cells, implying that NFκB activation was involved in LPS-induced CD40 expression.

Involvement of TLR4 in LPS-induced CD40 Expression

It's well established that LPS exerts its biological effects mainly but not entirely through the TLR4 complex and its co-receptors (Wong *et al.*, 2000; Beutler, 2002). By means of flow cytometry using anti-human TLR4 antibody, we measured TLR4 expression on THP-1 cells. This study showed that in addition to constitutive expression TLR4 could also be induced by LPS stimulation on THP-1 cells (Figure 5A). To examine the role of TLR4 in LPS-induced CD40 expression, THP-1 cells were pre-incubated with 10 μg/ml of isotype IgG or HTA125, a TLR4 neutralizing antibody (HTA125), for 2 h prior to LPS treatment for 24 h. As shown in Figure 5B, HTA125 partially but significantly blunted LPS-induced CD40 expression. These data suggested that TLR4 mediated LPS-induced CD40 expression on THP-1 cells. Since HTA125 is a weak TLR4 neutralizing antibody (Wang *et al.*, 2003), we next utilized two mouse macrophage cell lines, namely HeNC2 and GG2EE, to further define the involvement of TLR4 in LPS-induced CD40 expression. HeNC2 cells express wild-type TLR4 whereas GG2EE cells express mutated TLR4 that is functionally inactive. As shown in Figure 5C, LPS induced a dose-dependent increase in CD40 expression on both cell lines, but expression was significantly reduced in GG2EE versus HeNC2 cells. This line of evidence supported the notion that TLR4 participated in LPS-induced CD40 expression.

MAPKs Were not Required for LPS-induced NFκB

Since both MAPKs and NFκB were implicated in LPS-induced CD40 expression on THP-1 cells, this study further examined the possible interaction between these two pathways. Kinase activity inhibitors were administered to evaluate their effect on LPS-induced IκBα degradation and nuclear NFκB-DNA binding in THP-1 cells. As expected, in DMSO (vehicle)-pretreated cells, LPS induced a marked reduction of IκBα, but inhibitors for ERK kinase, JNK, and p38 kinase had minimal effect on LPS-induced IκBα degradation (Figure 6A). To further verify

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

this observation, a gel shift assay was performed to examine the effect of kinase inhibitors on LPS-induced NFκB-DNA binding. As expected, the positive control, TNFα (100ng/ml), induced a pronounced increase in NFκB-DNA binding (Figure 6B). Consistently, all three MAPK inhibitors had minimal effect on LPS-induced NFκB-DNA binding. These data demonstrated that MAPKs were not responsible for LPS-induced NFκB activation in THP-1 cells.

Interaction between TLR4 and MAPKs or NF.B in LPS-treated THP-1 cells

To examine the interaction between TLR4 and MAPKs or TLR4 and NFκB, THP-1 cells were pre-incubated with 10 μg/ml of isotype IgG or HTA125 for 2 h prior to 1000 ng/ml LPS treatment for 30 min. As shown in Figure 7, HTA125 blunted LPS-induced phosphorylation of ERK (Figure 7A), JNK (Figure 7B), and p38 (Figure 7C), and degradation of IκBα (Figure 7D), respectively. In addition, ERK phosphorylation and IκBα degradation were also examined in HeNC2 and GG2EE cells exposed to LPS. In consistent with the findings from THP-1 cells, LPS stimulation induced more pronounced phosphorylation of ERK and degradation of IκBα in HeNC2 cells than in GG2EE cells (Figure 7E and 7F). These results indicated that TLR4 was required for LPS-induced MAPK and NFκB activation. Furthermore, we investigated the effects of MAPKs and NFκB on LPS-induced TLR4 expression. Pretreatment of THP-1 cells with DMSO, MAPK or NFκB inhibitors significantly inhibited LPS-induced TLR4 expression (Figure 8), respectively. Therefore, these data suggested that interaction between TLR4 and MAPKs or NFκB participated in LPS-induced CD40 expression.

Discussion

Downstream signaling of CD40 ligation has been well characterized, which may involve TNF receptor associated factors (TRAF), NFκB, MAPKs, phophoinositide-3 kinase (PI3K), phospholipase C (PLC), and STAT3 and 6 (van Kooten and Banchereau, 2000). This study using human monocytic cells demonstrated that MAPKs and NFκB could also participate in the upstream events regulating LPS-induced CD40 gene expression.

The mitogen-activated protein kinases (MAPKs) are generally expressed in all cell types. LPS is a potent activator of all three MAPKs (Sweet and Hume, 1996). Previous studies have demonstrated that MAPK-mediated LPS effects were cell type specific and depended on the surface molecules studied. For example, both JNK and p38, but not ERK, were found to mediate LPS-induced up-regulation of CD80, CD83, CD86, and CD54, but not that of HLA-DR in human dendritic cells (Nakahara *et al.*, 2004). JNK, but not p38 and ERK, was found to involve in LPS-induced B7.1, IL-10 or CD44 expression in THP-1 cells (Ma *et al.*, 2001; Gee *et al.*, 2002; Lim *et al.*, 2005). Inconsistent effects of MAPKs on LPS-induced surface marker expression have been observed. For example, one study demonstrated that p38 kinase was required for LPS-induced upregulation of CD80, CD83, and CD86, but did not have any significant effect on the LPS-induced changes in HLA-DR, CD40, and CD1a expression in human monocyte-derived dendritic cells (Ardeshna *et al.*, 2000). The other study showed that p38, but not ERK, was involved in LPS- and TNFα-induced upregulation of CD1a, CD40, and HLA-DR in the maturation of $CD83^{-}$ dendritic cells derived from human blood monocytes (Arrighi *et al.*, 2001). In contrast, our study with three kinase-specific inhibitors demonstrated that all three MAPKs including ERK, JNK, and p38 kinase were involved in LPS-induced CD40 expression on THP-1 cells. It is not possible to make quantitative inferences regarding the relative involvement of MAPKs based solely on the efficacy of the different MAPK inhibitors in blocking CD40 expression. However, our finding that each of the inhibitors partially blocked CD40 expression induced by LPS implied a complex regulatory mechanism that included all of the MAPK intermediates examined. Furthermore, differential involvement of MAPKs has also been observed in human dendritic cell maturation and Th1/Th2 engagement

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(Nakahara *et al.*, 2006). In addition to MAPKs, NFκB was also shown to play a critical role in LPS-induced CD40 expression since inhibition of NFκB activation with Bay 11–7082 significantly blocked CD40 expression on THP-1 cells exposed to LPS. Distinct roles for the five NFκB molecules including p50, p52, RelA (p65), c-Rel, and RelB have been identified in dendritic cell function (Berges *et al.*, 2005). In this study we observed that p65 was mainly affected by LPS exposure in THP-1 cells using the supershift assay.

Both MAPKs and NFκB were shown to participate in LPS-induced CD40 expression in this study. However, the mechanisms responsible for MAPK-mediated CD40 transcription or LPS-induced NFκB activation remained elusive. NFκB has been proposed as an important transcription factor in LPS-induced CD40 expression in murine macrophages (Qin *et al.*, 2005). Analysis of the CD40 promoter demonstrates that NFκB regulatory elements is conserved between the human and mouse CD40 promoters. Within the CD40 promoter, there are four NFκB sites that are designated as dκB, mκB, m2κB, and pκB (Nguyen and Benveniste, 2002). In this study, LPS exposure was found to activate the NFκB signaling pathway, including IκBα phosphorylation, degradation, and NFκB-DNA binding in THP-1 cells. Recent studies have investigated the upstream signaling pathways responsible for LPS-induced NFκB activation. It was shown that LPS could induce NFκB activation through transforming growth factor β (TGFβ)–activated kinase (TAK1), which was a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family (Akira and Takeda, 2004). PI3K was also demonstrated to mediate LPS-induced NFκB activation through induction of p65 phosphorylation (Guha and Mackman, 2001). Although MAPKs were activated by LPS exposure in this study, inhibition of MAPKs with specific kinase inhibitors did not block LPS-induced IκBα degradation and NFκB-DNA binding, implying that these kinases were not involved in LPS-induced NFκB activation. Few studies have explored the signaling events that mediated MAPK-related CD40 transcription. MAPK pathways control gene expression in a number of ways including the phosphorylation and regulation of transcription factors, co-regulatory proteins and chromatin proteins (Whitmarsh, 2006). As described earlier, MAPKs had little effect on LPS-induced NFκB activation. Thus, MAPKs might regulate CD40 expression through other transcription factors, such as STAT1. STAT1 has been reported to regulate CD40 expression through the Gas element in the CD40 promoter (Lee *et al.*, 2007; Qin *et al.*, 2005). Previous studies have shown that p38 MAPK, but not ERK, was involved in plasmin-triggered DNA binding of STAT1 and subsequent expression of CD40 in human primary monocytes (Burysek *et al.*, 2002). Pretreatment of mouse microglia cells with selective antagonists of ERK1/2 and p38 MAPK counteracted guanosine inhibition on cytokine-induced CD40 expression and function as well as on STAT1 nuclear translocation (D'Alimonte *et al.*, 2007).

Analysis of the time course of LPS-induced CD40 expression on THP-1 cells revealed that upregulation of CD40 expression occurred 8 h following LPS treatment in this study. This latent process of CD40 expression may involve autocrine stimulation of cytokine production. LPS stimulation has been shown to induce expression of cytokines and chemokines in THP-1 cells or murine macrophages (Glue *et al.*, 2002; Harrison *et al.*, 2004; Harrison *et al.*, 2005; Qin *et al.*, 2005). These cytokines or chemokines may modulate LPS-induced CD40 expression on THP-1 cells as on other cell types (Nguyen *et al.*, 2000; Nguyen *et al.*, 2002; Qin *et al.*, 2005). We speculate that MAPKs and NFκB activation induced by LPS is associated with the expression and feedback action of these cytokines. On the one hand, MAPKs and NFκB could regulate expression of these cytokines; on the other hand, these cytokines may modulate CD40 expression via activating MAPKs and NFκB (Lim *et al.*, 2002; Gee *et al.*, 2003; Ma *et al.*, 2004; Lim *et al.*, 2005). In addition, the effect of cytokines on MAPKs and NFκB could be interrelated or independent. Such a scenario could explain the lack of inhibition of NFκB activation by inhibiting MAPKs.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

LPS is the major component of the outer membrane of Gram-negative bacteria. The immune response to microbial pathogens relies on both innate and acquired immunity. Innate immunity is determined by the interaction between potential pathogens and their cognate binding receptors, including the Toll-like receptor (TLR) family (Akira and Takeda, 2004; Beutler, 2004). At least 10 TLRs (TLR1-TLR10) recognize specific molecular patterns that are present in microbial components. Stimulation of different TLRs induces distinct patterns of gene expression, which not only leads to the activation of innate immunity but also instructs the development of antigen-specific acquired immunity. TLR4 has now been established as the main transmembrane receptor for LPS although it also recognizes other biological components (Poltorak *et al.*, 1998; Takeuchi and Akira, 2001; Palsson-McDermott and O'Neill, 2004). In this study, we observed that TLR4 was constitutively expressed and could also be induced by LPS exposure on THP-1 cells. Pretreatment of THP-1 cells with the TLR4 neutralizing antibody HTA125 blunted LPS-induced CD40 expression, implying that TLR4 mediated LPS-induced CD40 expression on THP-1 cells. This notion was further strengthened by the studies using murine macrophage cell lines, which showed that GG2EE cells expressing a functionally mutated TLR4, presented impaired CD40 expression as compared to HeNC2 cells with functional TLR4. It has recently been proposed that TLR4 uses two different signaling pathways, the MyD88-dependent and –independent pathways, to lead to NFκB activation. The former involves the adaptor proteins MyD88 and Mal/TIRAP pathway with direct NFκB activation, and the latter involves the adaptor proteins TRIF and TRAM pathway with late NFκB activation (Yamamoto *et al.*, 2003a; Yamamoto *et al.*, 2003b). Our studies suggested that TLR4-mediated CD40 expression operated mainly through a TLR4-MyD88-dependent pathway in LPS-treated THP-1 cells. First, NFκB was rapidly activated by LPS stimulation. Second, GG2EE cells expressing mutated nonfunctional TLR4 (P712H) demonstrated reduced levels of CD40 expression than HeNC2 cells expressing wild-type functional TLR4. TLR4 (P712H) was incapable of interaction with MyD88 (Rhee and Hwang, 2000). The fact that CD40 expression was partially inhibited by HTA125 and GG2EE cells expressed reduced CD40 suggested that TLR4-independent mechanisms might also play some role in LPS-induced CD40 expression (Triantafilou *et al.*, 2001). Interestingly, in addition to acting as the downstream effector of TLR4 activation, MAPKs and NFκB were also shown to regulate TLR4 expression. The interaction between TLR4 and MAPKs or NFκB might strengthen LPS-induced CD40 expression on THP-1 cells.

CD40-CD154 has emerged as a key signaling pathway for the function of B cells, monocytes, and dendritic cells (DC) in the immune system. LPS as it relates to CD40 expression, promotes maturation of immature myeloid DCs and naïve monocytes to mature DCs. Therefore, understanding the mechanisms underlying LPS-induced CD40 expression in immune cells will provide fundamental information for more precise control over communication between innate and adaptive immune responses in diverse diseases.

ACKNOWLEDGEMENTS

We greatly appreciate Dr. Samet for his review of this manuscript, and John Lay and Heather Wells for their technical assistance. This work was supported by United States Environmental Protection Agency Cooperative Agreement CR#829522; NIH grant HL062624

References

Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4:499–511.

Alexis NE, Lay JC, Almond M, Bromberg PA, Patel DD, Peden DB. Acute LPS inhalation in healthy volunteers induces dendritic cell maturation in vivo. J Allergy Clin Immunol 2005;115:345–350. [PubMed: 15696093]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Ardeshna KM, Pizzey AR, Devereux S, Khwaja A. The PI3 kinase, p38 SAP kinase, and NF-kappaB signal transduction pathways are involved in the survival and maturation of lipopolysaccharide-stimulated human monocyte-derived dendritic cells. Blood 2000;96:1039–1046. [PubMed: 10910920]

Arrighi JF, Rebsamen M, Rousset F, Kindler V, Hauser C. A critical role for p38 mitogen-activated protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide, TNF-alpha, and contact sensitizers. J Immunol 2001;166:3837–3845. [PubMed: 11238627]

Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S. The CD40 antigen and its ligand. Annu Rev Immunol 1994;12:881–922. [PubMed: 7516669]

Becker S, Soukup J. Coarse(PM(2.5–10)), fine(PM(2.5)), and ultrafine air pollution particles induce/increase immune costimulatory receptors on human blood-derived monocytes but not on alveolar macrophages. J Toxicol Environ Health A 2003;66:847–859. [PubMed: 12746131]

Benveniste EN, Nguyen VT, Wesemann DR. Molecular regulation of CD40 gene expression in macrophages and microglia. Brain Behav Immun 2004;18:7–12. [PubMed: 14651941]

Berges C, Naujokat C, Tinapp S, Wieczorek H, Hoh A, Sadeghi M, Opelz G, Daniel V. A cell line model for the differentiation of human dendritic cells. Biochem Biophys Res Commun 2005;333:896–907. [PubMed: 15963458]

Beutler B. TLR4 as the mammalian endotoxin sensor. Curr Top Microbiol Immunol 2002;270:109–120. [PubMed: 12467247]

Beutler B. Inferences, questions and possibilities in Toll-like receptor signalling. Nature 2004;430:257–263. [PubMed: 15241424]

Burysek L, Syrovets T, Simmet T. The serine protease plasmin triggers expression of MCP-1 and CD40 in human primary monocytes via activation of p38 MAPK and janus kinase (JAK)/STAT signaling pathways. J Biol Chem 2002;277:33509–33517. [PubMed: 12093796]

Ciccarelli R, Gee K, Lim W, Ma W, Nandan D, Diaz-Mitoma F, Kozlowski M, Kumar A. Differential regulation of CD44 expression by lipopolysaccharide (LPS) and TNF-alpha in human monocytic cells: distinct involvement of c-Jun N-terminal kinase in LPS-induced CD44 expression. J Immunol 2002;169:5660–5672. [PubMed: 12421945]

D'Alimonte I, Flati V, D'Auro M, Toniato E, Martinotti S, Rathbone MP, Jiang S, Ballerini P, Di Iorio P, Caciagli F. Guanosine inhibits CD40 receptor expression and function induced by cytokines and beta amyloid in mouse microglia cells. J Immunol 2007;178:720–731. [PubMed: 17202332]

Gee K, Lim W, Ma W, Nandan D, Diaz-Mitoma F, Kozlowski M, Kumar A. Tumor necrosis factor-alpha induces functionally active hyaluronan-adhesive CD44 by activating sialidase through p38 mitogen-activated protein kinase in lipopolysaccharide-stimulated human monocytic cells. J Biol Chem 2003;278:37275–37287. [PubMed: 12867430]

Glue C, Hansen JB, Schjerling P, Jinquan T, Poulsen LK. LPS-induced cytokine production in the monocytic cell line THP-1 determined by multiple quantitative competitive PCR (QC-PCR). Scand J Clin Lab Invest 2002;62:405–412. [PubMed: 12469895]

Guha M, Mackman N. LPS induction of gene expression in human monocytes. Cell Signal 2001;13:85–94. [PubMed: 11257452]

Harrison LM, van Haaften WCE, Tesh VL. Regulation of proinflammatory cytokine expression by Shiga Toxin 1 and/or lipopolysaccharides in the hman monocytic cell line THP-1. Infect Immun 2004;72:2618–2627. [PubMed: 15102770]

Harrison LM, van den Hoogen C, van Haaften WC, Tesh VL. Chemokine expression in the monocytic cell line THP-1 in response to purified shiga toxin 1 and/or lipopolysaccharides. Infect Immun 2005;73:403–412. [PubMed: 15618178]

He T, Tang C, Xu S, Moyana T, Xiang J. Interferon gamma stimulates cellular maturation of dendritic cell line DC2.4 leading to induction of efficient cytotoxic T cell responses and antitumor immunity. Cell Mol Immunol 2007;4:105–111. [PubMed: 17484804]

Hock BD, McKenzie JL, Patton NW, Drayson M, Taylor K, Wakeman C, Kantarjian H, Giles F, Albitar M. Circulating levels and clinical significance of soluble CD40 in patients with hematologic malignancies. Cancer 2006;106:2148–2157. [PubMed: 16598754]

Lee SJ, Qin H, Benveniste EN. Simvastatin inhibits IFN-{gamma}-induced CD40 gene expression by suppressing STAT-1{alpha}. J Leukoc Biol 2007;82:436–447. [PubMed: 17507688]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Lim W, Ma W, Gee K, Aucoin S, Nandan D, Diaz-Mitoma F, Kozlowski M, Kumar A. Distinct role of p38 and c-Jun N-terminal kinases in IL-10-dependent and IL-10-independent regulation of the costimulatory molecule B7.2 in lipopolysaccharide-stimulated human monocytic cells. J Immuno 2002;168:1759–1769.

Lim W, Gee K, Mishra S, Kumar A. Regulation of B7.1 costimulatory molecule is mediated by the IFN regulatory factor-7 through the activation of JNK in lipopolysaccharide-stimulated human monocytic cells. J Immunol 2005;175:5690–5700. [PubMed: 16237059]

Ma W, Gee K, Lim W, Chambers K, Angel JB, Kozlowski M, Kumar A. Dexamethasone inhibits IL-12p40 production in lipopolysaccharide-stimulated human monocytic cells by down-regulating the activity of c-Jun N-terminal kinase, the activation protein-1, and NF-kappa B transcription factors. J Immunol 2004;172:318–330. [PubMed: 14688340]

Ma W, Lim W, Gee K, Aucoin S, Nandan D, Kozlowski M, Diaz-Mitoma F, Kumar A. The p38 mitogen-activated kinase pathway regulates the human interleukin-10 promoter via the activation of Sp1 transcription factor in 18 lipopolysaccharide-stimulated human macrophages. J Biol Chem 2001;276:13664–13674. [PubMed: 11278848]

Miyazawa M, Ito Y, Yoshida Y, Sakaguchi H, Suzuki H. Phenotypic alterations and cytokine production in THP-1 cells in response to allergens. Toxicol In Vitro 2007;21:428–437. [PubMed: 17118622]

Mizel SB, Honko AN, Moors MA, Smith PS, West AP. Induction of macrophage nitric oxide production by Gram-negative flagellin involves signaling via heteromeric Toll-like receptor 5/Toll-like receptor 4 complexes. J Immunol 2003;170:6217–6223. [PubMed: 12794153]

Nakahara T, Moroi Y, Uchi H, Furue M. Differential role of MAPK signaling in human dendritic cell maturation and Th1/Th2 engagement. J Dermatol Sci 2006;42:1–11. [PubMed: 16352421]

Nakahara T, Uchi H, Urabe K, Chen Q, Furue M, Moroi Y. Role of c-Jun N-terminal kinase on lipopolysaccharide induced maturation of human monocyte-derived dendritic cells. Int Immunol 2004;16:1701–1709. [PubMed: 15477228]

Nguyen VT, Benveniste EN. IL-4-activated STAT-6 inhibits IFN-gamma-induced CD40 gene expression in macrophages/microglia. J Immunol 2000;165:6235–6243. [PubMed: 11086058]

Nguyen VT, Benveniste EN. Critical role of tumor necrosis factor-alpha and NF-kappa B in interferon-gamma-induced CD40 expression in microglia/macrophages. J Biol Chem 2002;277:13796–13803.

Palsson-McDermott EM, O'Neill LA. Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. Immunology 2004;113:153–162.

Park JH, Chang HS, Park CS, Jang AS, Park BL, Rhim TY, Uh ST, Kim YH, Chung IY, Shin HD. Association analysis of CD40 polymorphisms with asthma and the level of serum total IgE. Am J Respir Crit Care Med 2007;175:775–782. [PubMed: 17255560]

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998;282:2085–2088.

Qin H, Wilson CA, Lee SJ, Benveniste EN. IFN-{beta}-induced SOCS-1 negatively regulates CD40 gene expression in macrophages and microglia. Faseb J 2006;20:985–987. [PubMed: 16571771]

Qin H, Wilson CA, Lee SJ, Zhao X, Benveniste EN. LPS induces CD40 gene expression through the activation of NF-kappaB and STAT-1alpha in macrophages and microglia. Blood 2005;106:3114–3122. [PubMed: 16020513]

Rhee SH, Hwang D. Murine TOLL-like receptor 4 confers lipopolysaccharide responsiveness as determined by activation of NF kappa B and expression of the inducible cyclooxygenase. J Biol Chem 2000;275:34035–34040. [PubMed: 10952994]

Schonbeck U, Libby P. The CD40/CD154 receptor/ligand dyad. Cell Mol Life Sci 2001;58:4–43.

Shanmugam N, Kim YS, Lanting L, Natarajan R. Regulation of cyclooxygenase-2 expression in monocytes by ligation of the receptor for advanced glycation end products. J Biol Chem 2003;278:34834–34844. [PubMed: 12837757]

Su B, Karin M. Mitogen-activated protein kinase cascades and regulation of gene expression. Curr Opin Immunol 1996;8:402–411. [PubMed: 8793994]

Sweet MJ, Hume DA. Endotoxin signal transduction in macrophages. J Leukoc Biol 1996;60:8–26. [PubMed: 8699127]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Takeuchi O, Akira S. Toll-like receptors; their physiological role and signal transduction system. Int Immunopharmacol 2001;1:625–635. [PubMed: 11357875]

Tone M, Tone Y, Babik JM, Lin CY, Waldmann H. The role of Sp1 and NF-kappa B in regulating CD40 gene expression. J Biol Chem 2002;277:8890–8897. [PubMed: 11751910]

Triantafilou K, Triantafilou M, Dedrick RL. A CD14-independent LPS receptor cluster. Nat Immunol 2001;2:338–345. [PubMed: 11276205]

Van Kooten C, Banchereau J. CD40-CD40 ligand: a multifunctional receptor-ligand pair. Adv Immunol 1996;61:1–77. [PubMed: 8834494]

van Kooten C, Banchereau J. Functions of CD40 on B cells, dendritic cells and other cells. Curr Opin Immunol 1997;9:330–337. [PubMed: 9203418]

van Kooten C, Banchereau J. CD40-CD40 ligand. J Leukoc Biol 2000;67:2–17. [PubMed: 10647992]

Wang R, Stephens J, Lacy MJ. Characterization of monoclonal antibody HTA125 with specificity for human TLR4. Hybrid Hybridomics 2003;22:357–365. [PubMed: 14683595]

Wang T, Zhang X, Li JJ. The role of NF-kappaB in the regulation of cell stress responses. Int Immunopharmacol 2002;2:1509–1520. [PubMed: 12433052]

Whitmarsh AJ. Regulation of gene transcription by mitogen-activated protein kinase signaling pathways. Biochim Biophys Acta 2007;1773:1285–1298. [PubMed: 17196680]

Wong PM, Chugn SW, Sultzer BM. Genes, receptors, signals and responses to lipopolysaccharide endotoxin. Scand J Immunol 2000;51:123–127. [PubMed: 10722369]

Wu W, Jaspers I, Zhang W, Graves LM, Samet JM. Role of Ras in metal-induced EGF receptor signaling and NF-kappaB activation in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2002;282:L1040–L1048. [PubMed: 11943669]

Wu W, Silbajoris RA, Whang YE, Graves LM, Bromberg PA, Samet JM. p38 and EGF receptor kinase-mediated activation of the phosphatidylinositol 3-kinase/Akt pathway is required for Zn2+-induced cyclooxygenase-2 expression. Am J Physiol Lung Cell Mol Physiol 2005;289:L883–L889. [PubMed: 15980035]

Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 2003a;301:640–643. [PubMed: 12855817]

Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K, Akira S. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. Nat Immunol 2003b;4:1144–1150. [PubMed: 14556004]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

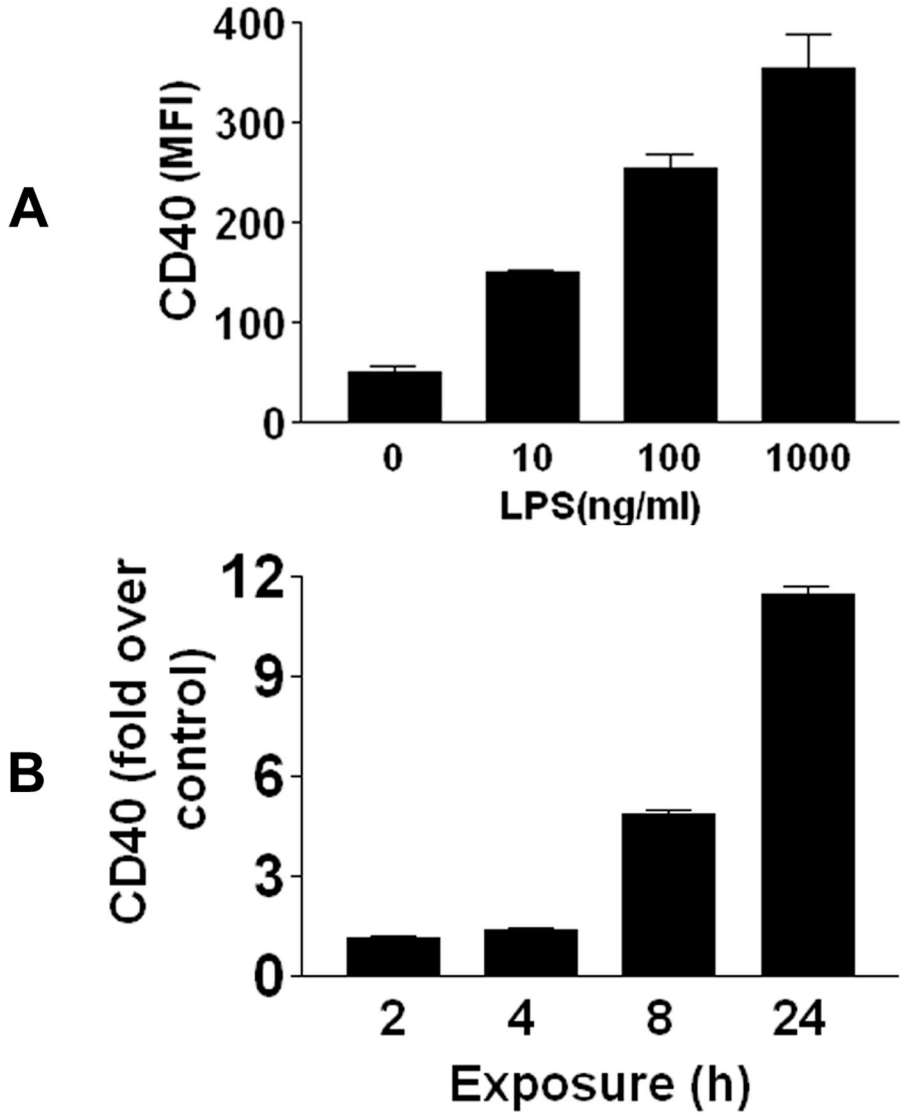

Figure 1.
LPS exposure results in increase in CD40 expression on THP-1 cells. A, THP-1 cells were exposed to 0–1000 ng/ml of LPS for 24 h. B, THP-1 cells were exposed to 1000 ng/ml of LPS for 2, 4, 8, and 24 h. CD40 expression was measured with flow cytometry using isotype and anti-CD40 antibodies, respectively, as described in Materials and Methods.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

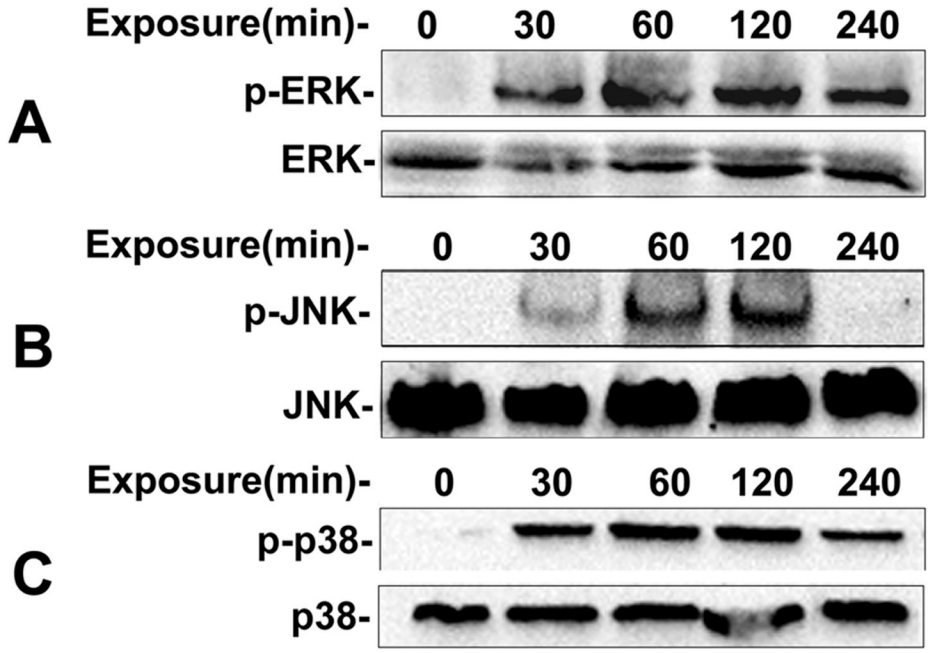

Figure 2.
LPS induces phosphorylation of MAPKs in THP-1 cells. THP-1 cells were exposed to 1000 ng/ml of LPS for 0, 30, 60, 120, and 240 min. Cells were lysed with RIPA buffer. Supernatants of cell lysates were subjected to SDS-PAGE and immunoblotting. Phosphorylated ERK (A), JNK (B), and p38 kinase (C) were detected using phospho-specific antibodies. Data shown are representative of three separate experiments.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

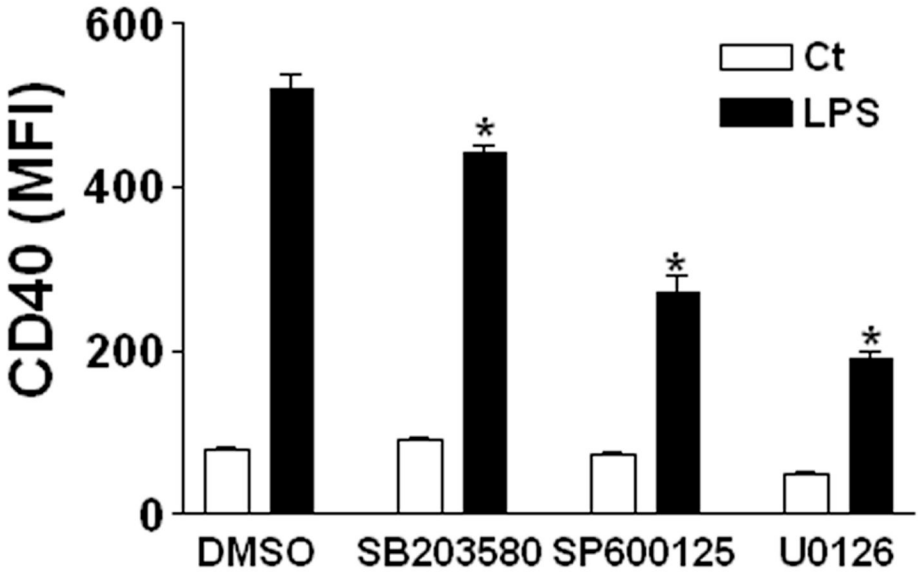

Figure 3.
MAPK inhibitors inhibit LPS-induced CD40 expression on THP-1 cells. THP-1 cells were pretreated with DMSO, 20 μM U0126, 20 μM SP600125, or 20 μM SB203580 for 30 min, respectively, prior to 1000 ng/ml LPS treatment for 24 h. CD40 expression was determined using flow cytometry. * $P<0.05$, compared to vehicle (DMSO) LPS.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

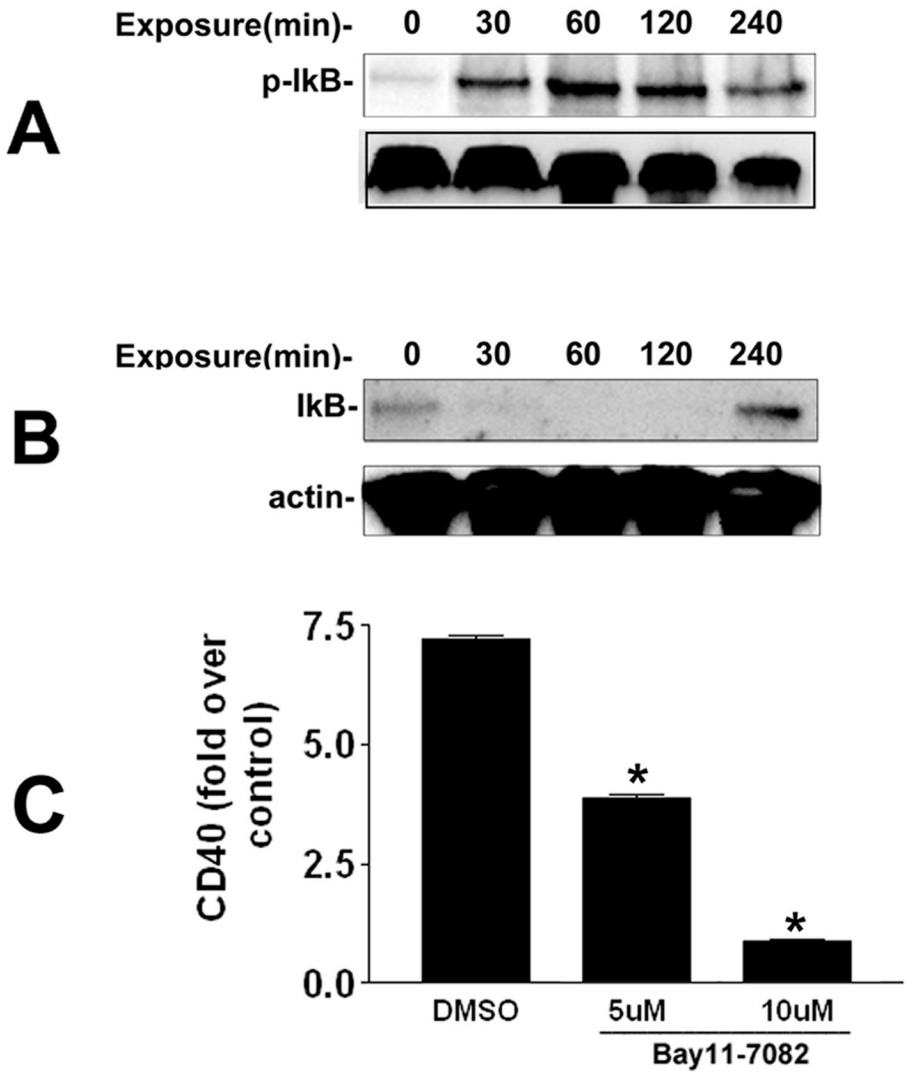

Figure 4.

NFκB activation is required for LPS-induced CD40 expression on human THP-1 cells. A, THP-1 cells were pretreated with 20 μM MG-125 for 30 min , then incubated with 1000 ng/ml LPS for 0, 30, 60, 120, and 240 min. Cells were lysed with RIPA buffer. Cell lysates were subjected to SDS-PAGE and immunoblotting. Phosphorylated IκBa was detected using phospho-specific anti-IκBα antibody. B, THP-1 cells without MG-125 pretreatment were incubated with 1000 ng/ml LPS for 0, 30, 60, 120, and 240 min. IκBα and actin protein levels were measured as described above. C, THP-1 cells were pretreated with DMSO, 5 μM or 10 μM Bay 11–7082 for 30 min prior to 1000 ng/ml LPS stimulation for 24 h, respectively. CD40 expression (MFI) was measured with flow cytometry. * $P<0.05$, compared to vehicle (DMSO) LPS.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

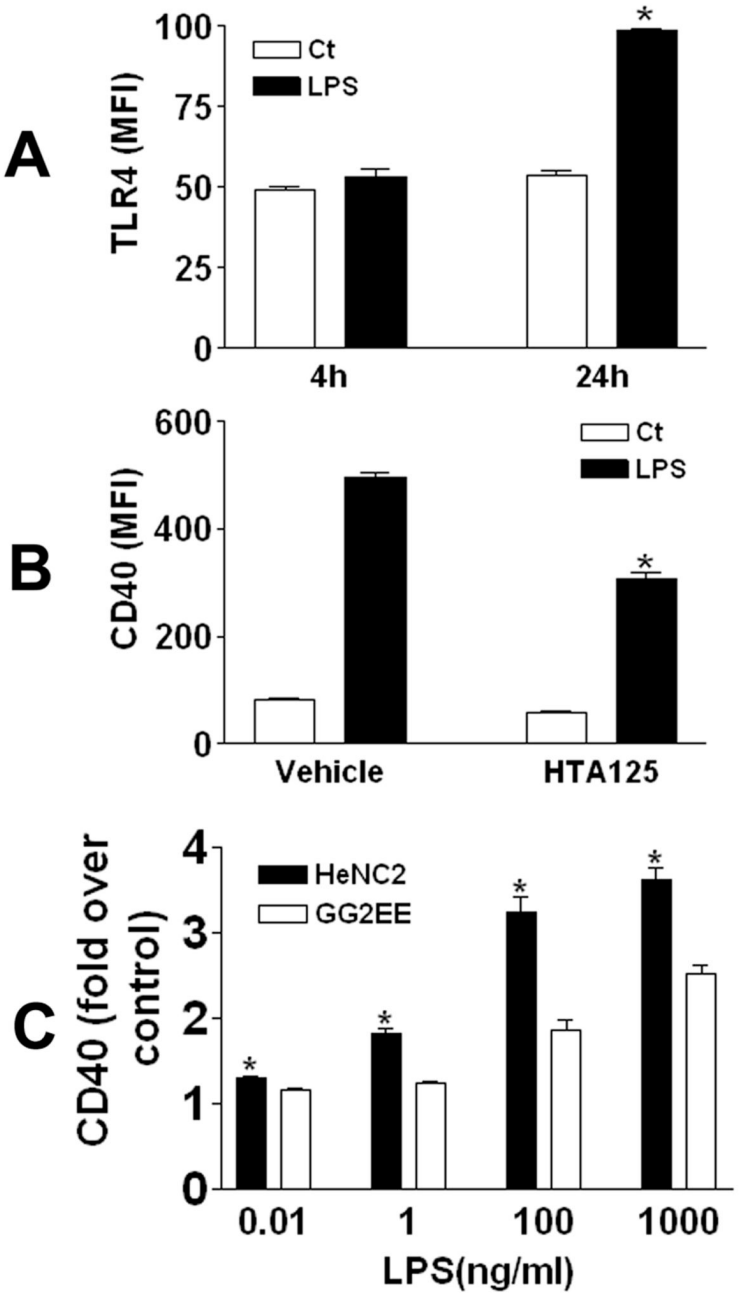

Figure 5.

TLR4 is involved in LPS-induced CD40 expression. A, THP-1 cells were treated with 1000 ng/ml LPS or control (PBS) for 4 h and 24 h. TLR4 was determined with flow cytometry using anti-TLR4 antibody (**P*<0.05 vs. control, 24 h). B, THP-1 cells were pretreated with HTA125 (10 μg/ml) or isotype IgG for 2 h prior to LPS (1000 ng/ml) treatment for 24 h. CD40 expression (MFI) was measured with flow cytometry (**P*<0.05 vs. vehicle LPS). C, Mouse macrophage cell lines HeNC2 and GG2EE were treated with 0.01, 1, 100, and 1000 ng/ml of LPS for 24 h. CD40 expression was measured with flow cytometry (* *P*<0.05, compared to GG2EE).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

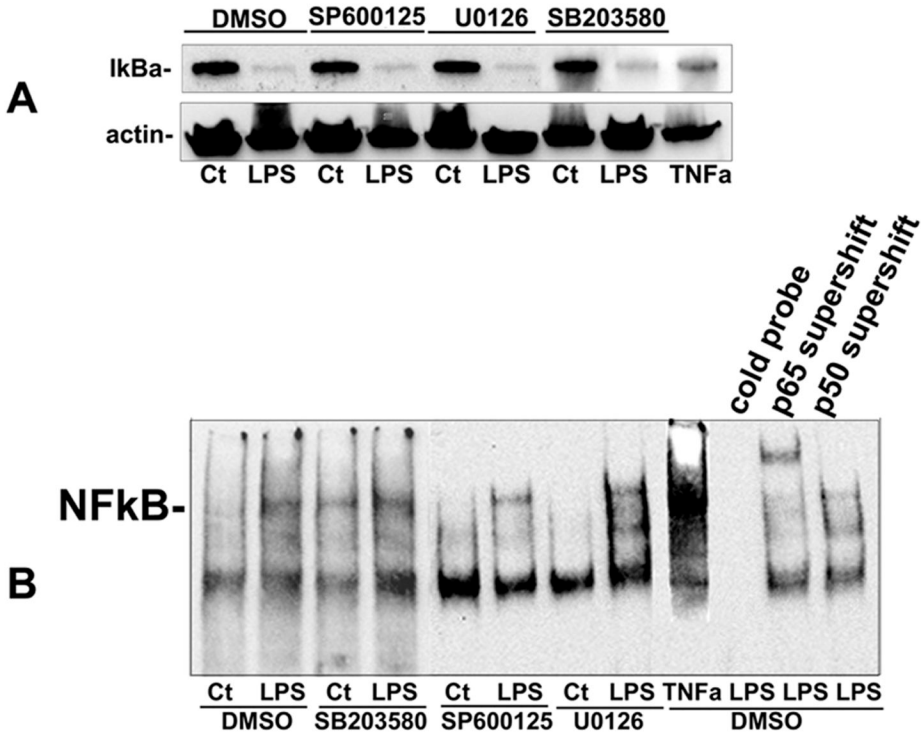

Figure 6.

MAPKs are not required for LPS-induced NFκB activation. THP-1 cells were pretreated with DMSO (vehicle), 20 μM U0126, 20 μM SP600125, or 20 μM SB203580 for 30 min, respectively, prior to 1000 ng/ml LPS treatment for 60 min. A, IκBα and actin protein levels were measured with immunoblotting using anti-IκBα and anti-actin antibodies. B, NFκB-DNA binding was detected using a gel shift kit following the manufacture's instruction. A supershift assay was conducted using concentrated anti-p65 and anti-p50 antibodies.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

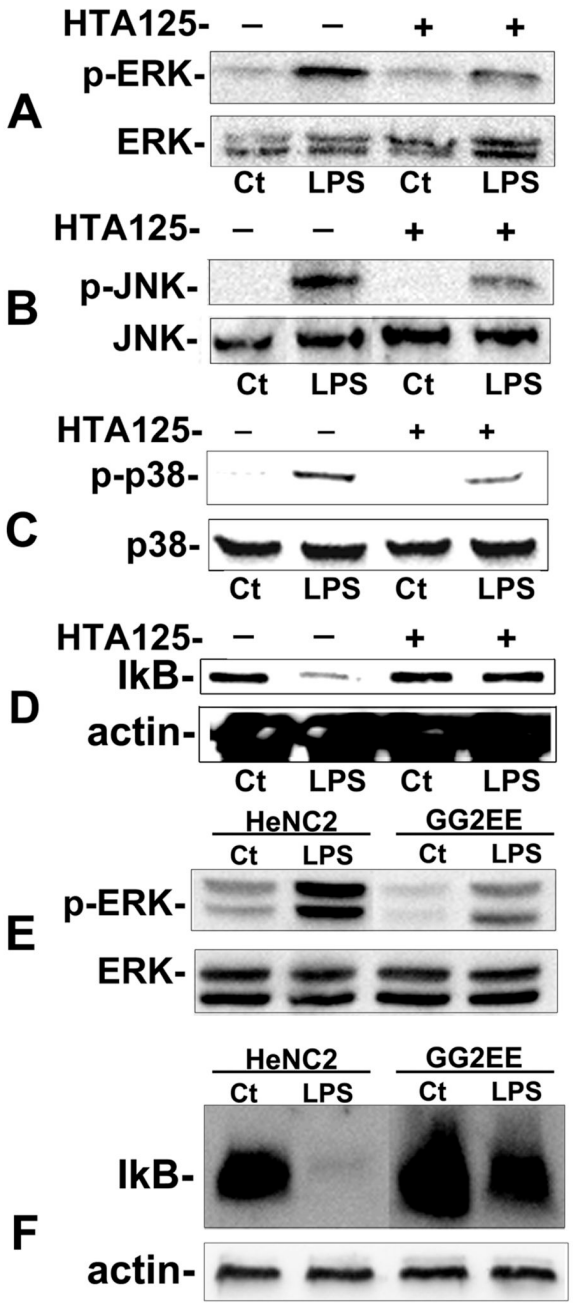

Figure 7.

TLR4 is involved in LPS-induced MAPK and NFκB activation. THP-1 cells were pretreated with HTA125 (10 μg/ml) or isotype IgG for 2 h prior to LPS (1000 ng/ml) treatment for 30 min. Phosphorylation of ERK (A), JNK (B), p38 (C), and IκBα protein levels (D) were determined with immunoblotting as described previously. Mouse macrophage cell lines HeNC2 and GG2EE were treated with 1000 ng/ml LPS for 30 min. Phosphorylation of ERK and IκBα protein levels (E and F) were measured with immunoblotting.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

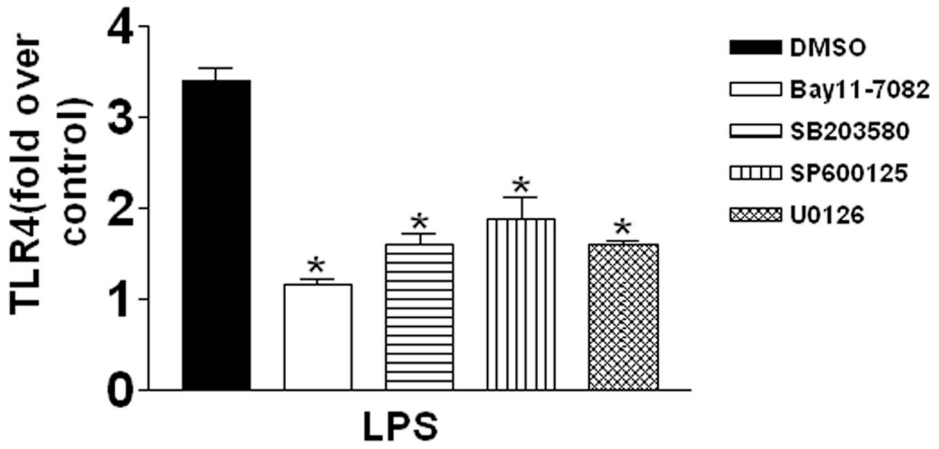

Figure 8.

MAPKs and NFκB are involved in LPS-induced TLR4 expression on THP-1 cells. THP-1 cells were pretreated with DMSO (vehicle), 20 μM U0126, 20 μM SP600125, 20 μM SB203580, or 10 μM Bay 11–7082 for 30 min, respectively, prior to 1000 ng/ml LPS treatment for 24 h. TLR4 expression was determined using flow cytometry. Levels of TLR4 expression induced by LPS were expressed as fold over control. * $P<0.05$, compared to vehicle (DMSO) LPS.